Gregory Wane, AI Australian Nature and General Photo Library

cue blooms

Graeme O'Neill applauds the capture of a master flowering gene.

When you're rooted to the spot, the natural state for all the world's flowering plants, it pays to be closely attuned to your environment. To germinate or flower at the wrong time can prove fatal.

In the equable environments of hundreds of laboratories around the world, the plant geneticist's 'green rat', *Arabidopsis thaliana*, will germinate at any time of year, then races through its life cycle, flowering at 30 days and setting seed within just 42 days.

In Sweden, the northern limit of its native range in Europe, the diminutive crucifer germinates beneath melting snow as temperatures begin to rise in late winter. It delays flowering until late spring, consigning its seeds to the soil in early summer.

Experiments have shown that the Swedish ecotypes won't flower until exposed to low winter temperatures: a phenomenon called vernalisation. And they delay flowering until daylength exceeds some critical minimum. Where laboratory strains of *Arabidopsis* send up flowering spikes from a small rosette of half a dozen leaves, the Swedish plants grow dozens of leaves before vernalisation and the lengthening spring days cue them to flower.

The adaptive value of the Swedish plants' inbuilt temperature and daylength sensors is obvious. They ensure that seed set occurs at the most propitious time of year, when the risk of lethal late-season frosts is minimal.

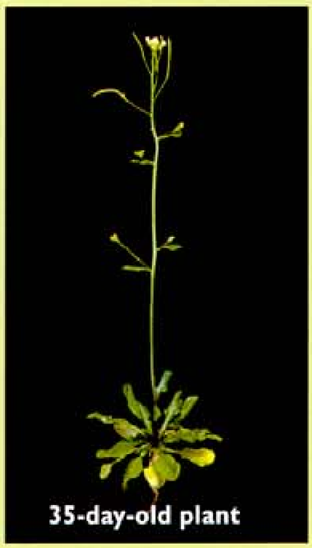
Like their Swedish cousins, several seedlings among Dr Candice Sheldon's latest crop of random mutants were slow to flower: they grew dozens of leaves and developed a hummock-like appearance before flowering at 70 days.

Sheldon, a member of Dr Liz Dennis's team at CSIRO Plant Industry, allowed the mutant plant to self-pollinate, to double-up the unknown gene that was delaying flowering. In the next generation of seedlings, some took 150 days to flower, and others still had not flowered 12 months later.

Dennis and her colleagues are gene hunters; they explore the *Arabidopsis* genome with a technique called transposon tagging. Transposons are mobile DNA elements that, when introduced into tissue-cultured cells, randomly insert themselves into chromosomes. Occasionally, a transposon lands within an anonymous gene, disrupting its activity and changing the way the plant looks, or the way it grows or responds to its environment. The transposon, now embedded in the gene, serves as a 'tag' that allows the researchers to locate and clone the gene, then explore its function.

It's now history that one of Sheldon's random shots had hit a rare genetic prize: the master gene that controls flowering. The gene, now known as *FLF* (Flowering Locus F), works in an unexpected way. Rather than initiating flowering, it suppresses flowering until other genes switch *FLF* off.

These genes remain anonymous, and it's not yet clear how they repress *FLF*, but they probably are key players in the systems that allow *Arabidopsis* to monitor its environment. One such mechanism monitors daylength



35-day-old plant

150-day-old *FLF* mutant plant

Left: *Arabidopsis* normally takes about 30 days to flower, and 42 to set seed.

Above: A crop of seedlings in which a gene later named *FLF* was disrupted produced several random mutants that were slow to flower. In the next generation flowering was delayed even further.

and in combination with the cold-treatment overcomes *FLF* in late spring, initiating flowering.

Sheldon and Dennis believe *FLF* is one of the master flowering genes, because it sits where all these pathways intersect. In effect, it plays gatekeeper, integrating data about the plant's developmental status, and the conditions in the external environment.

Only when all systems are 'go' does *FLF* switch off, setting in train the genetic programs that will transform fast-dividing, unspecialised meristem cells at the apex of the plant shoot – or shoots – into the intricate structures of flowers.

The diversity of flower-forming programs is enormous; every species has evolved its own unique combination of shape, colour and scent. But the gene that controls it all, *FLF*, is probably highly conserved in all flowering plants.

Now that they have identified *FLF*, Dennis and her colleagues hope to track back up through the pathways that suppress it, looking for ways of manipulating its activity. Farmers would welcome a way of delaying or advancing flowering in their crops to capitalise on seasonal conditions and premium markets. Commercial flower growers would prefer that their millions of chrysanthemums flowered in synchrony, on Mother's Day.

Doubtless farmers would also welcome a way of suppressing flowering completely. Like *Arabidopsis*, many agricultural weeds are quick to flower, and can squeeze several generations into a single growing season. A late winter spray that suppressed flowering could halt this rapid proliferation. Millions of Australians who spend spring and early summer racked by sneezing, and with streaming eyes, would welcome anything that prevented the perennial ryegrass infesting suburban lawns producing its potent pollen.

Dennis says the forestry industry would benefit from an anti-flowering compound. 'You don't want timber plantations to flower and set seed every year, you want all their resources directed into growing wood,' she says.

Eucalypt plantations invariably attract native birds and mammals that feed on nectar and pollen. Non-flowering plantations would avoid the carnage that accompanies clearfelling.

With their requirement for vernalisation – exposure to a period of winter cold – *Arabidopsis* ecotypes from northern Europe have something in common with fruit trees such as cherries and apples, and the winter wheats of colder regions of Europe and North America.

How does vernalisation work?

A decade ago, researchers became aware of the importance of a phenomenon called methylation in controlling gene activity in plants and animals. When the organism needs to shut down a gene, an enzyme called methyltransferase loads up its DNA with methyl (CH_3) molecules.

Plant Industry chief Dr Jim Peacock wondered if methylation might be the mysterious mechanism responsible for

The *FLF* gene integrates data about the plant's developmental status and external environmental conditions. When the *FLF* gene is switched off, the genetic programs that initiate flowering are set in train.

~16 days to flowering

less *FLF* product ←

~30 days to flowering

more *FLF* product →

>300 days to flowering

vernalisation. Perhaps exposure to cold stripped methyl molecules off the DNA to reactivate a suppressed gene in the flowering pathway for winter wheat?

His colleagues, Dr Dick Brock and Dr Jim Davidson, confirmed his hunch when they induced early flowering in a winter wheat by artificially vernalising it with a chemical demethylator, azacytidine.

'Nobody believed us when we suggested that methylation controlled vernalisation by suppressing the genes involved in flowering,' Dennis says.

It was that experiment that led Dennis and her colleagues into the search for the genes that control flowering. They set out to determine if flowering in *Arabidopsis* was also controlled by methylation.

Now they have shown that cold-shocking cold-tolerant ecotypes of *Arabidopsis* seedlings to simulate vernalisation suppresses the *FLF* gene's activity, initiating early flowering. And the same thing happens when the seedlings are demethylated with azocytidine.

The likely mechanism in vernalisation is that, when *Arabidopsis* germinates beneath the snow, the gene that controls the vernalisation-dependent flowering pathway is fully methylated and silent. Cold shock slowly demethylates the gene; by spring, it is fully demethylated, and it transmits the signal that switches off *FLF*, thus initiating flowering.

Peacock's predecessor as chief of Plant Industry, Dr Lloyd Evans, devoted much of his career to the search for 'florigen', an elusive elixir thought to trigger flowering.

His subject was the grass, *Lolium temulentum*, which stubbornly refuses to flower until a certain day in spring, when daylength exceeds some critical threshold. Evans, who still works in the division as an honorary research fellow, was able to induce early flowering in *Lolium* by treating its shoots with a the plant hormone, gibberellic acid, in winter, when daylength is normally too short to initiate flowering.

Evans' long-time colleague, Dr Rod King, says applying gibberellic acid (GA) to *Lolium* results in massive stem elongation, followed by flowering. The same response is seen in *Arabidopsis* where changing the daylength regime in a growth cabinet, from short days to long days, causes gibberellins to increase in the shoots: the leaves 'sit up', elongate and the plant flowers. King calls the gibberellins the plant's 'Viagra'.

Long day length causes a sharp rise in GA levels in the plants' tissues, but King says such experiments do not establish that gibberellic acid directly initiates flowering.

The events that occur between the application of the hormone (or its endogenous production in response to the light switch of increasing daylength) and the moment two days later when the plant initiates growth of the flowering spike, are hidden within a 'green box'

King and his colleagues are trying to identify what happens inside that green box. Their *Lolium* research had shown that gibberellins activate a member of the *MYB* family of developmental genes, which play key roles in plant organ development.

They have now shown that the *MYB* gene's protein binds to the promoter of another gene called *leafy*, which appears to be a master activator gene that regulates flower development, shoot elongation and leaf growth.

King and his colleagues have illuminated some of the pathway that allows *Arabidopsis* – at least, the Swedish ecotypes – to track increasing daylength, and cues the plants to produce flowers or leafy shoots from which their flowers will form in late spring.

So gibberellic acid activates *MYB*. The next question, says King, is how?


Lisa Coutts

Check genetic **facts** on line

A GUIDE to the science of gene technology, and a discussion of its benefits and risks, is available on the CSIRO website at http://genetech.csiro.au. The guide has been developed as part of the Federal Government's Biotechnology Australia initiative.

It covers topics such as the uses and regulation of gene technology in Australia, and outlines examples of the place of biotechnology in medicine, agriculture and the food industry. An extensive glossary of terms and links to other sites are also provided.

Abstract: Using a technique called transposon tagging, scientists have identified the master gene that controls flowering in plants. The gene, known as *FLF* suppresses flowering until switched off by other genes in response to environmental cues. The discovery raises the possibility of developing anti-flowering compounds for use in crop production and weed control. Other research has shed light on the pathway that allows the laboratory plant *Arabidopsis* to flower in response to increasing daylength. A process called methylation has been shown to control vernalisation (flowering at low temperatures) by suppressing the flowering genes.

Keywords: *Arabidopsis thaliana*, *Lolium temulentum*, flowers, flowering plants, plant physiology, transposon tagging, *FLF* (Flowering Locus F), gene.